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(71) Applicant ( <i>for all designated States except US</i> ):	STAZIONE ZOOLOGICA "ANTON DOHRN" [IT/IT]; Villa Comunale, 1, I-80121 Napoli (IT).		
(72) Inventors; and			
(75) Inventors/Applicants ( <i>for US only</i> ):	BOWLER, Chris [GB/GB]; Stazione Zoologica "Anton Dohrn", Villa Comunale, 1, I-80121 Napoli (IT). MUSTILLI, Anna, Chiara [IT/IT]; Stazione Zoologica "Anton Dohrn", Villa Comunale, 1, I-80121 Napoli (IT).		
(74) Agents:	BANCHETTI, Marina et al.; Ing. Barzani & Zanardo Roma S.p.A., Via Piemonte, 26, I-00187 Roma (IT).		
(54) Title:	NUCLEOTIDE SEQUENCES ENCODING THE TOMATO LIGHT HYPERSENSITIVE PHENOTYPE, ENCODED PROTEINS AND USES THEREOF		
(57) Abstract	<p>Nucleotide sequences of the tomato <i>TDET1</i> (<i>HP-2</i>) gene are described, which sequences, if modified, result in a light hypersensitive phenotype. Vectors and uses for the production of transgenic plants are also described and transgenic plants so obtained.</p>		

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NUCLEOTIDE SEQUENCES ENCODING THE TOMATO LIGHT  
HYPERSENSITIVE PHENOTYPE, ENCODED PROTEINS AND USES THEREOF

DESCRIPTION

The present invention relates to nucleotide sequences  
5 encoding the tomato light hypersensitive phenotype, encoded  
proteins and uses thereof.

In particular the present invention relates to  
nucleotide sequences encoding a protein, whose qualitative  
or quantitative modification and/or inhibition in plants  
10 induces high levels of carotenoids and/or flavonoids and/or  
chlorophylls, in comparison with wild-type plants; the  
invention also relates to the use of these nucleotide  
sequences for the production of engineered plants to be  
employed in the agro-industrial sector.

Light is a critical environmental signal that  
controls many aspects of plant growth and development. It  
is perceived by a sophisticated series of photoreceptors:  
the phytochromes, which absorb red and far red light, the  
cryptochromes, which absorb blue and UV-A light  
20 wavelengths, and the UV-B receptors (Mustilli and Bowler,  
1997). Together with endogenous hormonal signals, these  
photoreceptors regulate the developmental changes known as  
photomorphogenesis. Photomorphogenesis is defined as the  
influence of light on plant development and comprises leaf  
25 and chloroplast development and the regulation of  
photosynthetic apparatus components, by means of the  
coordinated expression of both nuclear and cytoplasmic  
genes. Moreover, due to the light response, photoprotectant  
pigments such as flavonoids are also produced. The  
30 modifications occurring during photomorphogenesis have been

characterized by studying light effects on *Arabidopsis* seedling development (von Arnim and Deng, 1996). Light-grown *Arabidopsis* seedlings display short hypocotyls, open and expanded cotyledons and the expression of light-regulated genes which are responsible for flavonoid and chlorophyll biosynthesis (e.g. chalcone synthase, *CHS*; chlorophyll A/B binding protein, *CAB*). Dark-grown seedlings display elongated hypocotyls, closed cotyledons and repression of light-regulated genes.

In higher plants, the phytochromes are encoded by a gene family (e.g. PHYA-E in *Arabidopsis*, Sharrock and Quail, 1989; Clack et al., 1994) and although they are the best characterized photoreceptors, relatively little is known about how the light signals perceived by phytochromes are transduced to the nucleus to activate the various developmental, physiological and molecular responses to light. Recently, biochemical studies using microinjection into cells of the phytochrome deficient *aurea* (*au*) tomato mutant, along with pharmacological studies in photomixotrophic soybean cell cultures, have implicated heterotrimeric G-proteins, cGMP, calcium and calmodulin as intermediates in phytochrome signal transduction pathways (Bowler and Chua, 1994; Mustilli and Bowler, 1997). In parallel, several genetic screens have been developed to identify mutants potentially affected in light signal transduction (Chamowitz and Deng, 1996). Most of the photomorphogenic mutants have been characterised in *Arabidopsis* and can be classified as either insensitive or constitutive mutants. Insensitive mutants display a light-blind elongated phenotype in the light. Some are mutated in the photoreceptors themselves, whilst others are presumed to encode positive regulators of light signal transduction

pathways (Chamovitz and Deng, 1996; Chory et al., 1996; Whitelam and Harberd, 1994). Conversely, constitutive de-etiolated mutants (e.g. *cop/det/fus/cpd*) display light grown morphologies when grown in the dark together, in some cases, with the inappropriate expression of light regulated genes such as *CAB* and *CHS* (Millar et al., 1994; Szekeres et al., 1996). The recessive nature of these mutations suggests that they are loss-of-function and that the wild-type genes are repressors of photomorphogenesis in darkness. However, although epistasis tests with phytochrome-deficient mutants have indicated that they function downstream of phytochrome, they are not specifically mutated in phytochrome signal transduction because many have altered tissue specificities as well as other additional phenotypes not directly related to light (Mayer et al., 1996; Chory and Peto, 1990; Millar et al., 1994; Szekeres et al., 1996). It is therefore not clear how COP/DET/FUS/CPD proteins function in the signal transduction pathways defined biochemically (Bowler and Chua, 1994).

A more targeted approach to identify specific components of signal transduction pathways specific for phytochrome could be the isolation of mutants with altered dynamics of light responses, rather than mutants with constitutive phenotypes in the absence of light. Several such light hypersensitive mutants have already been isolated in tomato (denoted *hp-1*, *hp-2*, *atv*, *Ip*; Kendrick et al., 1994). In particular, the recessive non-allelic *hp-1* and *hp-2* mutants have been characterized by their exaggerated light responsiveness, displaying higher anthocyanin levels (a flavonoid subgroup), shorter hypocotyls and more deeply pigmented fruits than wild-type plants. These mutants were

first identified in 1917 (Reynard, 1956) and in 1975 (Soressi, 1975), respectively. Recently, *hp-1<sup>w</sup>* (Peters et al., 1989) and *hp-2<sup>j</sup>* (Van Tuinen et al., 1997) mutants have been isolated and identified as new *hp-1* and *hp-2* alleles, 5 respectively. Because these phenotypes appear to be identical to those obtained by ectopic expression of phytochrome A (*PHYA*) in tomato (Boylan and Quail, 1989), it would appear that the *hp* mutation may affect fairly specifically phytochrome responses. The recessive nature of 10 the mutations, coupled with results from epistasis tests of *hp-1* with the phytochrome deficient tomato mutants *aurea* (*au*), *phyA* (*fri*), and *phyB* (*tri*), have suggested that *HP* genes encode negative regulators of light signal transduction mechanisms, acting downstream of both *PhyA* and 15 *PhyB* (Kerckhoffs et al., 1997). The fact that no counterparts of *hp* mutants have been isolated so far in *Arabidopsis*, along with the observation that in tomato anthocyanin production and the expression of photoregulated 20 genes (e.g., *CHS* and *CAB*) is strictly light-dependent, indicates the importance of *hp* mutants for studying phytochrome-dependent signal transduction. Furthermore, microinjection-based studies using the *au* tomato mutant have shown that tomato is an excellent model system to map 25 the role of individual components in the phytochrome activated signalling cascade (Bowler and Chua, 1994). Therefore the identification and characterization of *hp* genes is likely to be very important for studying the regulation of photomorphogenesis in plants.

The authors of the present invention have cloned the 30 tomato *HP-2* gene and have studied at the molecular level the role of the *HP-2* protein during the modulation of photomorphogenesis and fruit development. The authors have

found that the tomato *HP-2* gene exhibits high sequence homology with the *Arabidopsis DET1* gene, which belongs to the above described constitutive *COP/DET/FUS* mutant group. Therefore the tomato *HP-2* gene has been renamed *TDET1*.

5       The authors have used *Solanum lycopersicum* (tomato) species plants, but those skilled in the art will recognize that the cloning could be repeated with no inventive efforts with other plant species, as but not limited to pepper, eggplant, soybean, grape, melon, rice, carrot,  
10      spinach, citrus, pomaceae and ornamental species. The authors have cloned and sequenced the gene responsible for the tomato *hp* mutation (*high pigment*), which causes a light hypersensitive phenotype, thus enhancing carotenoid, and/or chlorophyll and/or flavonoid pigment levels. The gene is  
15      the first to be identified that causes such a mutant phenotype.

*hp* mutants potentially have a direct application in the agro-industrial sector, for generating tomato fruits with high carotenoid and/or flavonoid contents. In  
20      particular, in tomato fruits of *hp* mutants, a high content of the carotenoid lycopene as well as other carotenoids and flavonoids has been observed(Thompson, 1955; Yen et al., 1997). However, up to now the use of *hp* mutants in the agro-industrial sector, even if bred into various  
25      commercial varieties, has been impaired because of the fact that the *hp* mutation generates other undesirable phenotypes, such as reduced internode length and reduced plant vigour.

      The cloning of the *TDET1* gene and its use by means of  
30      gene transfer technologies offers considerable advantages with respect to conventional breeding techniques. It is possible to transfer genes suitable for agriculture between

different species and to inactivate and/or engineer genes of the same species, even only in specific plant tissues. Furthermore gene transfer is a much more rapid technique than conventional breeding.

5       The cloning of the *TDET1* gene now allows the production of engineered plants which exhibit all the favourable features of the *hp* mutation, with none of the undesirable side-effects. Such plants could belong to the *Solanum* genus, but those skilled in the art will recognize  
10      that, with no inventive effort, it is possible to transfer the gene, or parts thereof, subcloned in suitable expression vectors, into plants belonging to different genera, as e.g. but not limited to pepper, eggplant, soybean, grape, melon, rice, carrot, spinach, citrus,  
15      pomaceae and ornamental species.

It has been well recognized that a diet rich in lycopene and other carotenoids, or their administration in the form of pills, produce favourable effects on human health. As a matter of fact, carotenoids have antioxidant properties,  $\beta$ -carotene is a pro-vitamin A and lycopene is known to be an effective antitumoral agent (Bartley and Scolnik, 1995; Giles and Ireland, 1997; Hoffmann and Weisburger, 1997; Pappalardo et al., 1997; Pool-Zobel et al., 1997; Rock et al., 1997; Sharoni et al., 1997; Stahl and Sies, 1996). Therefore the engineered plants of the present invention can be advantageously utilized as a source of such compounds, either as fresh or processed foods or as nutraceuticals.

Furthermore *hp-2* mutants have high levels of  
30      flavonoids such as anthocyanins (Von Wettstein-Knowles, 1968), which are also considered to be excellent antioxidants and which exhibit in some cases antitumoral

properties (Fotsis et al., 1997; Rice-Evans et al., 1997). Furthermore some flavonoids exhibit a role in plant protection against pathogenic agents and UV light irradiation (Shirley, 1996).

5 Therefore the manipulation of *TDET1* activity can be used to modify carotenoid and/or flavonoid content in several plant species (e.g., tomato, pepper, eggplant, soybean, grape, melon, rice, carrot, spinach, citrus, and pomaceae) for biotechnological uses in both the biomedical  
10 and agro-industrial sectors.

Furthermore the manipulation of *TDET1* gene expression can be used to modify the anthocyanin and carotenoid content in ornamental species for the achievement of new colour variants (Griesbach, 1984).

15 In addition, because it has been recognized that a high content of carotenoids improves resistance to Norflurazon-type herbicides, a further application of the *TDET1* gene is the production of transgenic plants by selection using herbicides rather than antibiotic compounds  
20 (Misawa et al., 1993).

Furthermore in the same plant it is possible to combine a modified *TDET1* activity with mutations such as *rin*, *nor* and *Nr*, which interrupt the fruit ripening process, or with biosynthetic genes of the carotenoid  
25 biosynthesis pathway (Bartley and Scolnik, 1995), to obtain varieties exhibiting new qualitative characteristics for agro-industry.

The attainment of an *hp* mutant phenotype by means of a biotechnological approach can be carried out  
30 advantageously by means of the inhibition of *TDET1* activity. Currently the best method for reducing gene expression is through the introduction, by gene transfer,

of the antisense sequence of the same gene or part thereof under the control of appropriate regulatory sequences. The use of such techniques in plants has been carried out in several existing examples (Oeller et al., 1991; Penarrubia 5 et al., 1992), but those skilled in the art will recognize that alternative techniques can be used, without departing from the scope of the present invention.

Furthermore, by using specific vectors such as, for example, but not limited to, pE8mGFP4, which contains 10 regulatory sequences of the tomato *E8* gene (Fig. 9) (Deikman and Fischer, 1988), the inactivation of the *TDET1* gene can be specifically modulated in the tomato fruit. Those skilled in the art will recognize that other 15 regulatory sequences, e.g., originating from the polygalacturonase gene (*PG*) (Nicholass et al., 1995), can be used in the place of the *E8* gene promoter to obtain specific tissue modulation of *TDET1* gene expression.

Within the scope of the present invention the term "light hypersensitive phenotype" means reduced plant growth 20 associated with high levels of carotenoids and/or chlorophylls and/or flavonoids.

The term "protein or functional parts thereof, responsible for the light hypersensitive mutant phenotype" means an amino acid sequence which, if structurally or 25 otherwise altered, induces a light hypersensitive phenotype in a plant.

Therefore one object of the present invention is a nucleic acid comprising a nucleotide sequence encoding a protein, or functional parts thereof, which, if modified, 30 is responsible for the light hypersensitive mutant phenotype in *Solanum lycopersicum* plants, said phenotype including reduced plant growth associated with high levels

of carotenoids and/or chlorophylls and/or flavonoids. Nucleic acids encoding proteins which are homologous to proteins of the *Arabidopsis* COP/DET/FUS family, which when modified result in a light hypersensitive phenotype, are 5 within the scope of the present invention.

Preferably the nucleic acid comprises the nucleotide sequence encoding the TDET1 protein, or functional parts thereof. More preferably the nucleic acid comprises a nucleotide sequence encoding the protein having the amino 10 acid sequence of SEQ ID No. 2 or functional portions thereof. More preferably the nucleic acid has a nucleotide sequence comprised in SEQ ID No. 1, more preferably from nt. 149 to nt. 1720. Alternatively the nucleic acid has a nucleotide sequence complementary to SEQ ID No. 1, or parts 15 thereof.

In one aspect of the invention the nucleic acid of SEQ ID No. 1 comprises a mutation which is able to induce the light hypersensitive phenotype; preferably at least a C→T substitution in position 1640; alternatively the nucleic acid of SEQ ID No. 1 is deleted at least from nt. 20 1581 to nt. 1589.

A further object of the present invention is an expression vector including, under the control of an active and inducible plant promoter, the nucleic acid of the 25 invention. Preferably the promoter is active only in some plant organs, more preferably in fruits. Preferred vectors are able to drive the transcription of an antisense RNA, for example pBIN-E8-HP2-AS1 and pBIN-E8-HP2-AS2.

A further object of the invention is the use of the 30 vectors of the invention for producing transgenic plants which comprise the nucleic acid, under the control of specific regulating sequences, preferably in preselected

plant organs. Plants can belong to pepper, eggplant, soybean, grape, melon, rice, carrot, spinach, citrus, pomaceae and ornamental species.

A further object of the invention is a transgenic 5 plant, or parts thereof, achievable by transformation with the vectors of the invention. Plants can belong to pepper, eggplant, soybean, grape, melon, rice, carrot, spinach, citrus, pomaceae and ornamental species.

Plant genetic transformation techniques are known to 10 those skilled in the art and comprise, but are not limited to transformation by Agrobacterium, electroporation, microinjection, or bombardment with DNA coated particles (Christou, 1996).

In a further aspect the invention includes a protein, 15 or functional parts thereof, whose modification is responsible for the light hypersensitive phenotype in *Solanum lycopersicum* plants. Proteins homologous to the *Arabidopsis* COP/DET/FUS family are within the scope of the present invention, provided that, when modified, they 20 result in a light hypersensitive phenotype. Preferably the protein comprises the amino acid sequence of SEQ ID No. 2 or parts thereof.

In one aspect of the invention the protein comprises a modification which is able to induce the light 25 hypersensitive phenotype; preferably at least a modification in its C-terminal portion; more preferably a replacement of proline at position 498, most preferably serine as a substitute for proline; alternatively a deletion of at least one amino acid, preferably at least of 30 three amino acids, more preferably from aa. 478 to aa. 480 of SEQ ID No. 2.

The present invention will be described by reference to explanatory, but not limiting, examples, wherein reference will be made to the following figures:

Figure 1. Phenotypes of wild type, *hp-2*, and *hp-2<sup>j</sup>* tomato seedlings grown in the presence or in the absence of light. a) Hypocotyl length of seedlings grown in the absence (Dark) and in the presence of light (Light). b) Content of anthocyanins in the hypocotyls (dashed bars) and in the cotyledons (empty bars) of seedlings grown in the light. Data represent average values from ten seedlings. Following a three day pre-germination period in darkness, seedlings were grown either in darkness (Dark) or in a 16 hour light, 8 hour dark photoperiod (Light) for 6 days.

Figure 2. *TDET1* (*HP-2*) cDNA nucleotide sequence (SEQ ID NO. 1). The sequence encoding the *TDET1* protein is depicted in capital letters. The sequence of the RFLP CT151 marker covers nt. 830 to nt. 2000.

Figure 3. Amino acid sequence of the *TDET1* (*HP-2*) protein (SEQ ID No. 2). Amino acids are depicted in the standard one letter code for amino acids.

Figure 4. Exon-intron structure of the tomato *TDET1* (*HP-2*) gene and sites of mutations in *hp-2* and *hp-2<sup>j</sup>* mutant alleles. Stippled boxes indicate exons. Exons correspond to the following sequences of SEQ ID No. 1 (Figure 2): Exon 1: nt. 149 to nt. 220; Exon 2: nt. 221 to nt. 648; Exon 3: nt. 649 to nt. 877; Exon 4: nt. 877 to nt. 1012; Exon 5: nt. 1013 to nt. 1081; Exon 6: nt. 1082 to nt. 1138; Exon 7: nt. 1139 to nt. 1219; Exon 8: nt. 1220 to nt. 1384; Exon 9: nt. 1385 to nt. 1480; Exon 10: nt. 1481 to nt. 1580; Exon 11: nt. 1581 to nt. 1720; b) Donor and acceptor splice sites of intron 10 from the wild type and the *hp-2* mutant. Brackets indicate splice sites, dots indicate internal intron

sequence (not shown), and amino acids are shown in the one letter code. The asterisk in the *hp-2* sequence denotes the site of the mutation.

Figure 5. Alignment of the amino acid sequences of 5 *S. lycopersicum* TDET1, *Arabidopsis* DET1, and deduced mammalian EST amino acid sequences with homology to DET1 (determined using Clustal method; Higgins and Sharp, 1988). The putative bipartite NLS is overlined. The amino acids missing in the *hp-2* mutant are indicated with asterisks, 10 and the amino acid substitution in *hp-2J* is denoted by a plus sign. The mammalian sequences are a compilation of derived amino acid sequences from mouse and human ESTs (GenBank accession numbers AA756238, AA236057, AA050184, and W64359). Boxed residues indicate conserved amino acids, 15 dashes indicate arbitrary insertions.

Figure 6. Effect of cytokinins on wild type tomato seedlings. (a) and (b) Hypocotyl length (cm) and anthocyanin content (per mg fresh weight [FW]), respectively, of wild type tomato seedlings grown in the presence of 0, 1, 5, 20, 20 100, and 500 µg/L benzoaminopurine. Seedlings were grown at 25 °C for 5 days in absolute darkness (Dark) or in a 16-hr light 8-hr dark photoperiod (Light). In (a) values are the mean of 10 seedlings. Highly similar results were obtained with the cytokinin zeatin (data not shown).

Figure 7. Phenotype of the *au hp-2* double mutant 25 phenotype. Hypocotyl length (cm) and anthocyanin accumulation (per seedling) in wild type, *au*, *hp-2*, and the *au hp-2* double mutant are shown. Seedlings were grown at 25 °C for 6 days in a 16-hr light 8-hr dark photoperiod. 30 Values are the mean of 15 seedlings, and the experiments were repeated three times.

Figure 8. Gene expression in wild type, *hp-2*, and *hp-2<sup>j</sup>* seedlings. Seedlings were grown at 25 °C for 5 days in absolute darkness (D), followed by 2 days in continuous white light (L). The RNAs were extracted from whole 5 seedlings (dark) or from cotyledons (C) and hypocotyls (H) (light). Modifications in gene expression in *hp-2* and *hp-2<sup>j</sup>* seedlings compared with wild type seedlings (wt) are shown to be principally light-dependent. Ten microgram samples of 10 total RNA were loaded on gels and analyzed for expression of *CHS1* (*CHS*), *CAB6* (*CAB*), *PR1-1b1* (*PR*) genes following RNA gel blotting. 30S rRNA is shown as a control for loading.

Figure 9. Binary plasmids to be transferred to plants.

#### Materials and Methods

##### 15 Plant material and growth conditions

The *hp-2* and *hp-2<sup>j</sup>* exaggerated photoresponse mutants, the *au hp-2* double mutant, and the corresponding wild type tomato seeds (*Solanum lycopersicum* cv Money Maker) were kindly provided by R. E. Kendrick and M. Koornneef 20 (Wageningen Agricultural University, the Netherlands). Seeds were surface sterilized and directly sown in magenta boxes (Sigma) containing 4.3 g/L Murashige-Skoog salts (Sigma) and 0.8 % agar. After 2 days pregermination in darkness, seedlings were grown at 25° C either in a 16 hour 25 light, 8 hour dark photoperiod or in continuous dark, as appropriate. For cytokinin treatment, seeds were sown in the presence of different concentrations of benzylaminopurine (Sigma).

##### Anthocyanin assay

30 Anthocyanins were extracted from cotyledons, hypocotyls, and whole seedlings with 0.5 mL acidified (1% HCl) methanol for 48 hr in darkness with shaking. The

extracts were separated by the addition of 0.4 mL of H<sub>2</sub>O and 1 mL of chloroform, followed by centrifugation for 5 min at 3,000 rpm. The absorbance of the upper phase was determined spectrophotometrically at 535 nm (*A*<sub>535</sub>), and the 5 anthocyanin content was calculated as (*A*<sub>535</sub>)/mg fresh weight or (*A*<sub>535</sub>)/seedling.

#### Carotenoid and Chlorophyll assay

In order to determine the content of carotenoids and chlorophyll in the tomato fruit, pericarp sections of 10 unripe (25 days after pollination) or ripe (50 days after pollination) fruit were weighed and incubated for 48 hours at 65°C in DMSO (dimethyl sulfoxide) in the absence of light. The contents of carotenoids and chlorophylls were determined by HPLC and values are reported as µg/g fresh 15 weight.

#### DNA and RNA Extraction

DNA and RNA extractions from leaf samples were carried out according to Dellaporta et al. (1983) and Verwoerd et al. (1989), respectively.

RNA gels (10 µg per lane) were blotted onto Hybond N+ membranes (Amersham) and hybridized with random primed probes (see below). Hybridization was performed for 24 hr at 50°C in phosphate buffer (7% SDS, 0.5M Na<sub>2</sub>HPO<sub>4</sub>, pH 7.2, 1mM EDTA), followed by 20-min washes in 40mM NaPO<sub>4</sub> pH 7.2, 25 1% SDS, and 1mM EDTA. Probes were the tomato chalcone synthase gene *CHS1* (O'Neill et al., 1990), chlorophyll a/b binding protein gene *CAB6* (Piechulla et al., 1991) and pathogenesis-related protein gene *PR-1b1* (Tornero et al., 1997). All RNA gel blots were repeated at least twice and 30 using different samples.

#### Isolation and sequence of genomic clones, cDNAs and PCR products

5' amplification of cDNA was performed using the RACE system (Gibco/BRL) on total tomato leaf RNA with the oligonucleotide 5'-CATCAACACTGCCAAC-3' (SEQ ID No. 3), derived from the CT151 RFLP marker sequence. A 0.8-kb 5 Polymerase Chain Reaction (PCR) product, containing the 5' end of the *TDET1* cDNA, was obtained after two different amplification reactions with TaqI polymerase (Perkin Elmer) using two CT151-derived nested primers: 5'-  
10 GAAAGCAGCCGTTGCT-3' (SEQ ID No. 4) and 5'-AGTCATCATCTTACGGC-3' (SEQ ID No. 5), with the provided anchor primer (Gibco/BRL). The 0.8kb PCR fragment was directly sequenced on both strands with thermosequenase (Amersham).

Total cDNA from wild type tomato (cv. Money Maker) 15 and the corresponding *hp-2* and *hp-2<sup>j</sup>* mutants were obtained by reverse transcription using avian myeloblastosis virus reverse transcriptase (Promega) of poly(A) mRNA isolated from leaves using oligo(dT) Dynabeads (Dynal). From these samples the *TDET1* (*HP-2*) cDNA sequence was PCR amplified 20 with the specific oligonucleotides:

5'-GTATGATTCACTAGTTAATGCTGCTGAAAG-3' (SEQ ID No. 6) and  
5'-CCCATACTAGTCGTCTGGCACTCTATCAAG-3' (SEQ ID No. 7), using the Expand High Fidelity system (Boehringer Mannheim). Subsequently the amplification product was subcloned in 25 pBluescript as a Spel fragment and 4 independent clones were sequenced on both strands.

Tomato genomic libraries in  $\lambda$ -DASH and  $\lambda$ -FIXII (kindly provided, respectively, by Jim Giovannoni, Texas A&M University, College Station, TX, and the Tomato Genome Center, Rehovot, Israel) were screened using standard 30 methods (Sambrook et al., 1989) with a  $^{32}$ P-labelled CT151 fragment as a probe. Overlapping fragments from different

recombinant phages were subcloned in pBluescript SK+ for sequencing of both strands.

The 5'-GAAGGTAATTTATATTAAACATAGAATAGA-3' (SEQ ID No. 8) and 5'-GTGATTCTAGGTTGATTCATCTAGA-3' (SEQ ID. No. 9) oligonucleotides were used to amplify the *HP-2* gene 3' sequence from genomic DNA of the *hp-2* mutant. The 1,300 bp amplification product was directly sequenced with the primer 5'-CAAATCGGTAACATAT-3' (SEQ ID No. 10) using a Thermosequenase kit (Amersham).

10 Binary plasmids for plant transformation

DNA fragments containing the tomato fruit-specific E8 promoter (Deikman and Fischer, 1988) were PCR amplified from *S. lycopersicum* total DNA with the following nucleotides: 5'-GGGGAAGCTTTCACGAAATCGGCCCTTA-3' (SEQ ID No. 11) and 5'-CCCGGATCCTTCTTTGCACTGTGAATGATTAG-3' (SEQ ID No. 12). The 1.2kb amplification products were subcloned as HindIII-BamHI fragments in the binary expression vector pmGFP4, derived from pBI121 (Bevan, 1984; Jefferson et al., 1987; Haseloff et al., 1997) in place of the 35S promoter, obtaining the plasmid pE8mGFP4. The mGFP4 gene was then excised and the inverted or complementary sequence of the *HP-2* gene, or parts thereof, was inserted, as BamHI-SacI fragments, obtaining the plasmid pBIN-E8-HP2-AS1 and pBIN-E8-HP2-AS2, respectively (Figure 9).

25 Results

*hp-2* mutant phenotypes

*hp-2* mutant seedlings exhibited an exaggerated light response with respect to wild type seedlings. Particularly, hypocotyl length was reduced (Figure 1a) and the content of 30 anthocyanin pigments in the cotyledons was higher than in the wild type (Figure 1b). Such phenotypes were strictly dependent on the presence of light; in fact in the absence

of light the length of hypocotyl was not considerably different to the wild type (Figure 1a) and anthocyanins were not produced (data not shown). The *hp-2<sup>j</sup>* mutant exhibited a stronger phenotype than the *hp-2* mutant, with reference both to hypocotyl length and the content of anthocyanins (Figure 1).

Fruits obtained from plants of *hp-2* and *hp-2<sup>j</sup>* mutants also exhibited exaggerated light responses. In the unripe fruits from *hp-2* and *hp-2<sup>j</sup>* mutants the content of chlorophyll was about five times higher than in fruits from wild-type plants, whereas in mature fruits the total content of carotenoids was about twice that of fruits from wild-type plants (Table 1).

Table 1

Chlorophyll and carotenoid content of tomato fruits from wild type, *hp-2* and *hp-2<sup>j</sup>* mutants.

Genotype	Total Chlorophylls (ripe fruit) μg/g fresh weight	Total Carotenoids (unripe fruit) μg/g fresh weight
wild type (Money Maker)	20	60
<i>hp-2</i>	90	124
<i>hp-2<sup>j</sup></i>	115	137

The Phenotype of the Tomato *hp-2* Mutant is Caused by Mutation in *DET1*

The *hp-2* mutation has been mapped previously using restriction fragment length polymorphism (RFLP) analyses of a segregating population derived from a cross between the *hp-2* mutant (*S. lycopersicum*) and *S. pennellii*. Mapping data with a second backcross (BC-2) population indicated a position close to the centromere of chromosome 1, within a

cluster of several RFLP markers (Balint-Kurti et al., 1995; Van Tuinen et al., 1997; Broun and Tanksley, 1996). The authors of the present invention have found that the CT151 RFLP marker (Tanksley et al., 1992), included in this 5 cluster, had high homology with the *Arabidopsis DET1* gene, mutations in which were previously identified as causing the deetiolated phenotype of *det1* mutants.

Comparison of CT151 with *Arabidopsis DET1* revealed that CT151 has homology to the 3' end of *DET1*. The authors 10 of the present invention isolated a full-length cDNA encoding *TDET1* using 5' rapid amplification of cDNA ends (Loh et al., 1989). Subsequently, the *TDET1* gene was isolated from genomic libraries in λ-DASH and λ-FIXII. Alignment of the *TDET1* cDNA and genomic sequences revealed 15 the presence of 10 introns, which is similar to the number found in *Arabidopsis* (Figure 4a). Nine introns are located in the same positions as those of the *Arabidopsis DET1* gene, whereas intron 2 of *TDET1* is not present in the *Arabidopsis* homolog (Figure 4a) (Pepper et al., 1994). 20 Comparative protein sequence analysis between *Arabidopsis DET1* and *TDET1* show 81.3% similarity and 74.8% identity (Figure 5). No major differences are present between the sequences, except for a small deletion of 16 amino acids at the centre of *TDET1*, suggesting that the tomato and 25 *Arabidopsis* genes are true homologs. A *DET1* homolog is not present in the yeast genome or in any prokaryotic genome sequenced to date (data not shown). However, mouse and human expressed sequence tags (ESTs) with homology to *DET1* have been identified (Figure 5), as well as a *Drosophila* 30 homolog.

Considering the presence of a nuclear localisation signal (NLS) (Robbins et al., 1991) in the *Arabidopsis DET1*

amino acid sequence, and observing that the DET1-GUS chimeric protein is localised in the nucleus (Pepper et al., 1994), DET1 in *Arabidopsis* may be responsible for the transcriptional repression of photomorphogenesis in the 5 absence of light. Considering the homology with HP-2 it is possible for the latter to have an analogous function in tomato. The TDET1 protein has been found to be localized in the nucleus (data not shown).

TDET1-encoding cDNAs from the wild type, and *hp-2* and 10 *hp-2<sup>j</sup>* mutants were amplified with specific primers from leaf mRNA. Amplified fragments were subcloned in pBluescript and 4 independent clones were sequenced on both strands. In *hp-2* the authors identified a substitution involving T in the place of C in exon 11 (nt. 1640) that 15 causes a replacement of proline by serine in the protein C-terminal region (aa. 498) (Figure 4a). In the *hp-2* mutant the authors identified an alternative splicing site for intron 10, which causes a three amino acid deletion (Gly, Pro, Glu) at the start of exon 11, in the second NLS domain 20 of the protein (aa. 478-480) (Figure 4a). To find the mutation site which was responsible for the alternative splicing, the authors sequenced the 3' end of the TDET1 gene from the *hp-2* mutant, using a fragment obtained by PCR amplification of genomic DNA. A replacement of AG by TG in 25 the consensus 3' splice junction of intron 10 was identified (Figure 4b). These results demonstrate that the "high pigment" phenotype of the *hp-2* and *hp-2<sup>j</sup>* tomato mutants is caused by mutation of the TDET1 gene mutation.

Sequencing of the TDET1 cDNAs derived from the *hp-2* 30 mutant showed that the mutation produces two different splicing products from intron 10 (Figure 4b), suggesting the presence of a limited amount of wild-type TDET1 protein

and that the *hp-2* mutant is not an inactive allele. This is consistent with the observation that the *hp-2* mutant allele is weaker than the *hp-2<sup>j</sup>* allele (Figure 1 and Table 1). Whether the *hp-2<sup>j</sup>* mutation is null awaits further analysis, 5 although the mutated proline residue in the *hp-2<sup>j</sup>* mutant is conserved in both the plant sequences. However, because the *hp-2* mutation is situated in the C-terminal domain of TDET1, as is the mutation in the *Arabidopsis det1-5* mutant allele, which has a clearly de-etiolated phenotype (Pepper 10 et al., 1994), it is apparent that the *hp-2* mutant phenotypes are not comparable with the *det1* mutant phenotype in *Arabidopsis*. Furthermore, clearly weak alleles of *det-1* in *Arabidopsis*, eg. *det1-1*, display visible dark phenotypes.

15 Comparison of Tomato *hp-2* and *Arabidopsis det1* Mutants

As is clear from the results presented, *hp-2* mutants are phenotypically different from *det1* mutants. Most conspicuously they do not display dark phenotypes, such as reduced hypocotyl length, opened apical hooks, or enlarged cotyledons, whereas these were selection criteria for the 20 isolation of *det* and *cop* in *Arabidopsis* (Chory et al., 1989; Deng et al., 1991). Conversely, *det1* mutants can even develop true leaves and floral buds in prolonged darkness. No such phenotypes can be observed in *hp* tomato seedlings 25 grown in the dark.

*Arabidopsis det1* mutants are also characterized by high level expression of light-regulated genes such as *CHS* and *CAB* in the dark, whereas in the light, *CHS* and *CAB* gene expression are similar to that observed in wild-type plants 30 (Chory et al., 1989). To examine the effects of *hp-2* and *hp-2<sup>j</sup>* mutations on gene expression, the authors have performed RNA gel blot analysis of light-regulated gene

expression using *CHS* and *CAB* gene fragments as probes. In agreement with the weak phenotypes of *hp-2* and *hp-2j* mutant seedlings grown in darkness, no dramatic alteration of *CHS* and *CAB* gene expression was observed. Nonetheless, *CHS* and 5 *CAB* mRNA levels were slightly higher in the *hp-2j* allele when compared with *hp-2* and wild-type seedlings (Figure 8). Interestingly, *CHS* mRNA appeared to be of a slightly smaller size than that found in light-grown material, perhaps indicating a differential light-dependent splicing. 10 Consistent with the exaggerated photoresponsiveness of *hp-2* and *hp-2j* mutants, *CHS* mRNA levels were significantly enhanced compared with wild-type seedlings following light irradiation for 48 hr (Figure 8). *hp-2j* seedlings contained higher expression levels of *CHS* than *hp-2*, and *CHS* mRNA was 15 particularly abundant in hypocotyls. In contrast to *CHS*, *CAB* mRNA levels were higher in cotyledons than in hypocotyls, and were slightly lower in light-exposed *hp-2* and *hp-2j* mutant seedlings when compared to the wild type (Figure 8). In summary therefore, in tomato *hp-2* mutants 20 dark expression of *CHS* and *CAB* genes is only very slight and deregulation of gene expression is essentially light-dependent. This is in strong contrast to the *CHS* and *CAB* gene deregulation characteristics of *Arabidopsis det1* mutants (Pepper et al., 1994).

25 *det1* seedlings have been also reported to display a strongly enhanced expression of stress-related genes such as those encoding pathogenesis-related (PR) proteins and glutathione reductase (Mayer et al., 1996). To examine whether this was also the case in *hp-2* and *hp-2j* mutants, 30 RNA gel blots were hybridized with a probe encoding tomato *PR-1b1* (Tornero et al., 1997). Although hypocotyls of *hp-2* seedlings reproducibly displayed *PR-1b1* gene expression at

slightly higher levels compared to wild-type seedlings, this effect was very weak compared to that observed in *Arabidopsis det1* mutants and has never been observed in *hp-2j* mutants (Figure 8).

Despite these above-mentioned differences, plastid development in darkness in the cotyledons of *hp-2* and *hp-2j* seedlings was found by the authors to be similar to that observed in *Arabidopsis det1* mutants (data not shown; Chory et al., 1989).

10 Cytokinin can Phenocopy the *hp* Mutant

Previous observations in *Arabidopsis* have shown that a *det1* phenotype can be phenocopied by the exogenous application of cytokinin (Chory et al., 1994).

To determine the effects of cytokinin treatment in tomato, the authors treated wild-type seedlings with different concentrations of cytokinin in the dark and in the light (Figure 6). Cytokinin was found not to phenocopy a *det1* mutation in tomato. In dark-grown seedlings, although hypocotyls are shorter in the presence of cytokinin, apical hook opening, cotyledon expansion, and anthocyanin biosynthesis were not observed (Figure 6), even after prolonged periods (up to three weeks) (data not shown). However, in the light, cytokinin can phenocopy the *hp* mutation: seedlings displayed shorter and thicker hypocotyls and accumulated high levels of anthocyanin (Figure 6).

These results therefore indicate that at least as far as the effects of cytokinin are concerned, the *hp* mutation in tomato is equivalent to the *det1* mutation in *Arabidopsis*. Such a supposition is consistent with the fact that no constitutive de-etiolated mutants have heretofore been identified in tomato and *CHS* gene expression and

consequent biosynthetic build-up of anthocyanin pigments is strictly dependent on the light, whereas in *Arabidopsis* anthocyanins can be produced also in the absence of light (Mustilli and Bowler, 1997).

5     Double Mutant Analyses

Double mutant analysis with *det1* and phytochrome-deficient mutants in *Arabidopsis* indicate that the *det1* mutation is completely epistatic to photoreceptor mutations (Chory, 1992). This has been interpreted as meaning that 10 *DET1* acts downstream of phytochrome. To determine the relationship between *TDET1* and phytochrome function, we examined the effects of the *hp-2* mutation in the *aurea* mutant background, a phytochrome chromophore-deficient mutant (Terry and Kendrick, 1996). In contrast to its 15 counterpart double mutant in *Arabidopsis*, *au hp-2* is similar to the single mutant *au*. For example, hypocotyls are elongated and anthocyanin accumulation is limited (Figure 7). Although in genetic terms this result therefore infers that *au* is epistatic to *hp-2*, the authors nonetheless consider it more likely that *TDET1* acts 20 downstream of phytochrome, as proposed for *Arabidopsis*, but that its activity as a negative regulator is strictly dependent upon the presence of active phytochrome. This requirement is clearly not observed in *Arabidopsis* (Chory, 25 1992). The small but significant reduction in hypocotyl length and the small increase in anthocyanin found in the *au hp-2* double mutant compared with *au* (Figure 7) is likely therefore to be a result of hypersensitivity caused by the . *hp-2* mutation towards the low amounts of functional 30 phytochrome present in the *au* mutant, which is estimated to be about 3% wild-type levels (Adams et al., 1989; Parks et al., 1987). Therefore the *hp-2* phenotype in tomato is

strictly dependent on the presence of phytochrome, whereas in *Arabidopsis* the *det1* mutation is independent of the presence or absence of functional photoreceptors.

The absence of de-etiolated mutants in tomato and the strict light- and phytochrome- dependence of the *hp* mutant phenotype may suggest that in tomato *TDET1* function is either redundant or that the signal transduction pathways regulating its activity operate in different ways in the two plants (Mustilli and Bowler, 1997). Because by Southern analysis the authors have shown that in tomato, like in *Arabidopsis* (Pepper et al., 1994), only one *DET1* homologous gene is present (data not shown), the latter hypothesis seems to be more likely.

Based upon the author's findings, it is likely that only a few key regulators will be found to be responsible for controlling light responses in all higher plants. Therefore it would be very interesting to clone other tomato genes encoding proteins homologous to COP/DET/FUS and examine whether modification of their activity also results in light hypersensitive phenotypes.

#### Binary plasmids for modulation of *TDET1* activity

In order to generate tomato plants with an *hp-2* phenotype specifically in the fruit, the authors constructed plasmids which are able to produce the *TDET1* antisense RNA sequence, or portions thereof, only in the fruit by using the regulatory sequences of the tomato *E8* gene (Figure 9).

#### Bibliography

- Adamse, P., et al. (1989) Photochem. Photobiol. 50, 107-111.
- Balint-Kurti, P.J., Jones, D.A., and Jones, J.D.G. (1995) Theor. Appl. Genet. 90, 17-26.

- Bartley, G.E. and Scolnik, P.A. (1995) Plant Cell 7, 1027-1038.
- Bevan, M. W. (1984) Nucl. Acids Res. 12, 8711-8721.
- Bowler, C. and Chua, N-H. (1994) Plant Cell 6, 1529-1541.
- 5 - Boylan, M.T. and Quail, P.H. (1989) Plant Cell 1, 765-773.
- Broun, P. and Tanksley, S.D. (1996) Mol. Gen. Genet. 250, 39-49.
- Chamovitz, D.A. and Deng, X.-W. (1996) Crit. Rev. Plant 10 Sci. 15, 455-478.
- Chory, J (1992) Development, 115, 337-354.
- Chory, J., et al. (1996) Proc. Natl. Acad. Sci. USA 93, 12066-12071.
- Chory, J., et al. (1989) Cell 58, 991-999.
- 15 - Chory, J. and Peto, C. (1990) Proc. Natl. Acad. Sci. USA 87, 8776-8780.
- Chory, J., et al. (1994) Plant Physiol. 104, 339-347.
- Chistou, P. (1996) Trends Plant Sci., 1, 423-431.
- Clack., T., Mathews, S. and Sharrock, R.A. (1994) Plant 20 Mol. Biol. 25, 413-427.
- Deikman, J. and Fischer, R.L. (1988) EMBO J. 7, 3315-3320.
- Dellaporta, S. L., Wood, J. and Hicks, J.B. (1983) Plant Mol. Biol. Reporter 1, 19-21.
- 25 - Deng, X.-W., Caspar, T. and Quail, P.H. (1991) Genes & Dev. 5, 1172-1182.
- Fotsis, T., et al. (1997) Cancer Res. 57, 2916-2921.
- Giles, G. and Ireland, P. (1997) Int. J. Cancer 10, 13-17.
- 30 - Griesbach, R. J. (1984) J. Hered. 75, 145-147.
- Haseloff, J., et al. (1997) Proc. Natl. Acad. USA 94, 2122-2127.

- Higgins, D.G., and Sharp, P.M. (1988) Gene 73, 237-244.
- Hoffmann, I and Weisburger, J.H. (1997) Cancer Epidemiol. Biomarkers Prev. 6, 643-645.
- Jefferson, R.A., Kavanagh, T.A. and Bevan M.W. (1987) Embo J. 6, 3901-3907.
- Kendrick, et al. (1994) Biochem. Soc. Symp. 60, 249-256.
- Kerckhoffs, L.H.J., et al. (1997) J. Plant Physiol. 50, 578-587.
- Loh, E.Y., et al. (1989) Science 243, 217-220.
- 10 - Mayer, R., Raventos, D. and Chua, N.-H. (1996) Plant Cell 8, 1951-1959.
- Millar, A.J., McGrath, R.B. and Chua, N.-H. (1994) Annu. Rev. Genet. 28, 325-349.
- Misawa, N., et al. (1993) Plant J. 4, 833-840.
- 15 - Mustilli, A.C. and Bowler, C. (1997) EMBO J. 16, 5801-5806.
- Nicholass, F. J., et al. (1995) Plant Mol. Biol. 28, 423-435.
- Oeller, P.W., et al. (1991) Science 254, 437-439.
- 20 - O'Neill, S.D., et al. (1990) Mol. Gen. Genet. 224, 279-288.
- Pappalardo, G, et al. (1997) Eur. J. Clin. Nutr. 51, 661-666.
- Parks, B.M., et al. (1987) Plant. Mol. Biol. 9, 97-107.
- 25 - Penarrubia, L., et al. (1992) Plant Cell 4, 681-687.
- Pepper, A., et al. (1994) Cell 78, 109-116.
- Peters, J.L., et al. (1989) J. Plant Physiol. 134, 661-666.
- 30 - Piechulla, B., et al. (1991) Mol. Gen. Genet. 230, 413-422.
- Pool-Zobel, B.L., et al. (1997) Carcinogenesis 18, 1847-1850.

- Reynard, G.B. (1956) Tomato Genet. Coop. Report 6, 22.
- Rice-Evans, C.A., Miller, N.J. and Paganga, G. (1997) Trends Plant Sci. 2, 152-159.
- Robbins, J., et al. (1991) Cell 64, 615-623.
- 5 - Rock, C.L., et al. (1997) Cancer Epidemiol. Biomarkers Prev. 6, 617-623.
- Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual. (Cold Spring Harbour, N.Y., Cold Spring Harbor Laboratory Press).
- 10 - Sharoni, Y., et al. (1997) Cancer Detect. Prev. 21, 118-123.
- Sharrock, R.A. and Quail, P.H. (1989) Genes & Dev. 3, 1745-1757.
- Shirley, B.W. (1996) Trends Plant Sci. 1, 377-382.
- 15 - Soressi, G.P. (1975) Tomato Genet. Coop. Report 25, 21-22.
- Stahl, W. and Sies, H. (1996) Arch. Biochem. Biophys. 336, 1-9.
- Szekeres, M., et al. (1996) Cell 85, 171-182.
- 20 - Tanksley, S.D., et al. (1992) Genetics, 132, 1141-1160.
- Terry, M.J., and Kendrick, R.E. (1996) J. Biol. Chem. 271, 21681-21686.
- Thompson, A.E. (1995) Science 121, 896-897.
- Tornero, P., et al. (1997) Mol. Plant Microbe Interact. 25 10, 624-634.
- Van Tuinen, A., et al. (1997) Theor. Appl. Genet. 94, 115-122.
- Verwoerd, T.C., Decker, B.M. and Hoekema, A. (1989) Nucl. Acids Res. 17, 2362.
- 30 - von Armin, A. and Deng, X.-W. (1996) Annu. Rev. Plant Physiol. Plant Mol. Biol. 47, 215-243.
- Von Wettstein-Knowles, P. (1968) Hereditas 60, 318-346.

- Whitelam, G.C. and Harberd, N.P. (1994) Plant, Cell and Environ. 17, 615-625.
- Yen, H.C., et al. (1997) Theor. Appl. Genet. 95, 1069-1079.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## 5 (i) APPLICANT:

- (A) NAME: Stazione Zoologica "Anton Dohrn"
- (B) STREET: Villa Comunale
- (C) CITY: Naples
- (E) COUNTRY: Italy

10 (F) POSTAL CODE (ZIP): 80121

(ii) TITLE OF INVENTION: Nucleotide sequences encoding the tomato light hypersensitive phenotype, encoded proteins and uses thereof

15 (iii) NUMBER OF SEQUENCES: 12

## (iv) COMPUTER READABLE FORM:

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- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
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## (2) INFORMATION FOR SEQ ID NO: 1:

## 25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2000 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA to mRNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 149..1720

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

	CTTCCCTCTT AGACTTTATC GATCCTAATT CGAGCCCTCC TTTTTTCAAT CAATTATCAA	60
	TTTAGTCCTA CTGCGATTTT GATATGTATG ATTACACAATT TTAATGCTGC TGAAAGCAAT	120
	TATATAAAAG CTGAAACATT TTGCACTG ATG TTC AAA ACT AAC AAT GTT ACC	172
	Met Phe Lys Thr Asn Asn Val Thr	
5	1	5
	GCC AGG CTT TTT GAG CGC CAG ATT TGC ACC CCT GCT CCT GGC ACC AGC	220
	Ala Arg Leu Phe Glu Arg Gln Ile Cys Thr Pro Ala Pro Gly Thr Ser	
	10 15 20	
10		
	ATC CAT CGT GCC AGA AGA TTT TAT GAG AAC GTT GTA CCA AGT TAT ACC	268
	Ile His Arg Ala Arg Arg Phe Tyr Glu Asn Val Val Pro Ser Tyr Thr	
	25 30 35 40	
15		
	ATA TAC GAT GTT GAA TGT CCC GAC CAT TCA TTT CGC AAG TTC ACG GAT	316
	Ile Tyr Asp Val Glu Cys Pro Asp His Ser Phe Arg Lys Phe Thr Asp	
	45 50 55	
20		
	GAC GGT CTA TAT TTT GTA AGT TTC AGC CGA AAC CAT CAG GAT CTG GTT	364
	Asp Gly Leu Tyr Phe Val Ser Phe Ser Arg Asn His Gln Asp Leu Val	
	60 65 70	
25		
	GTT TAT AGA CCA ACA TGG CTG ACA TTT TCC TGC AAA GAA GAA GAT TGT	412
	Val Tyr Arg Pro Thr Trp Leu Thr Phe Ser Cys Lys Glu Glu Asp Cys	
	75 80 85	
30		
	GAT ACT CAT GAT CTT CCT TTG AAA GCT AGA AAG TTT GAG AGC TTC TTC	460
	Asp Thr His Asp Leu Pro Leu Lys Ala Arg Lys Phe Glu Ser Phe Phe	
	90 95 100	
	ACA CAG TTG TAC AGT GTT ACT CTT GCT TCT AGT GGG GAA CTT ATA TGC	508
	Thr Gln Leu Tyr Ser Val Thr Leu Ala Ser Ser Gly Glu Leu Ile Cys	
	105 110 115 120	

	AAA GAT TTC TTT CTC TAT ATG GAG AGC AAC CAA TTT GGA CTC TTT GCA		556
	Lys Asp Phe Phe Leu Tyr Met Glu Ser Asn Gln Phe Gly Leu Phe Ala		
	125	130	135
5	ACT TCA ACT GCA CAA ATT CAT GAT GCA CCT CCT ACT GGA GGG GCA ATT		604
	Thr Ser Thr Ala Gln Ile His Asp Ala Pro Pro Thr Gly Gly Ala Ile		
	140	145	150
10	CAG GGA GTC CCT TCA GTT GAA AAA ATA ACT TTC CAC CTT TTG AGG TTG		652
	Gln Gly Val Pro Ser Val Glu Lys Ile Thr Phe His Leu Leu Arg Leu		
	155	160	165
15	GTG GAT GGA GCT ATA CTT GAC GAA AGG GTT TTC CAC AAT GAT TAT GTT		700
	Val Asp Gly Ala Ile Leu Asp Glu Arg Val Phe His Asn Asp Tyr Val		
	170	175	180
20	AAT TTG GCA CAT AGC ATT GGT GCT TTC TTG TAT GAT GAT TTG CTT GCT		748
	Asn Leu Ala His Ser Ile Gly Ala Phe Leu Tyr Asp Asp Leu Leu Ala		
	185	190	195
	200		
25	ATA GTG TCT CTT CGT TAT CAA AGA ATA CAC ATC CTT CAG ATC AGA GAT		796
	Ile Val Ser Leu Arg Tyr Gln Arg Ile His Ile Leu Gln Ile Arg Asp		
	205	210	215
30	TCT GGA GAT CTT GTT GAT GTA CGA GCA ATT GGG GAA TTC TGC CGT GAA		844
	Ser Gly Asp Leu Val Asp Val Arg Ala Ile Gly Glu Phe Cys Arg Glu		
	220	225	230
35	GAT GAT GAA CTT TTT CTC AAT TCC AAT TCC CAG GTG CTT GTA AAT CAT		892
	Asp Asp Glu Leu Phe Leu Asn Ser Asn Ser Gln Val Leu Val Asn His		
	235	240	245
	GTT GGA AAT GGT TTT CAT CAT AGT CTG CCT CAA TCA GAA ACT TCT TTC		940
	Val Gly Asn Gly Phe His His Ser Leu Pro Gln Ser Glu Thr Ser Phe		
	250	255	260

	CTG AGC GGT ATA AAG CAA CGG CTG CTT TCA TAT ATA TTT CGA GGT ATA	988
	Leu Ser Gly Ile Lys Gln Arg Leu Leu Ser Tyr Ile Phe Arg Gly Ile	
265	270	275
		280
5	TGG AAT GAA GCT GAC CAA ACC ATG AGA GTG CAG TGC CTG AAG AAG AAG	1036
	Trp Asn Glu Ala Asp Gln Thr Met Arg Val Gln Cys Leu Lys Lys	
	285	290
		295
10	TTT TAC TTC CAC TTT CAA GAT TAC ATT GAC TTG ATT ATC TGG AAG GTG	1084
	Phe Tyr Phe His Phe Gln Asp Tyr Ile Asp Leu Ile Ile Trp Lys Val	
	300	305
		310
15	CAG TTT TTG GAC CGA CAT CAC CTG TTG ATC AAG TTT GGC AGT GTT GAT	1132
	Gln Phe Leu Asp Arg His His Leu Leu Ile Lys Phe Gly Ser Val Asp	
	315	320
		325
	GGT GGG GTA TCC CGA AAT GCT GAC ATC CAT CCT TCT TTT TTT GCT GTT	1180
	Gly Gly Val Ser Arg Asn Ala Asp Ile His Pro Ser Phe Phe Ala Val	
	330	335
		340
20	TAC AAT ATG GAG ACT ACT GAA ATT GTT GCA TTT TAT CAG AAC TCA GCC	1228
	Tyr Asn Met Glu Thr Thr Glu Ile Val Ala Phe Tyr Gln Asn Ser Ala	
	345	350
		355
		360
25	GAT GAG CTT TAT TTC TTG TTC GAG CTG TTC AGC GAC CAT TTT CAC GTT	1276
	Asp Glu Leu Tyr Phe Leu Phe Glu Leu Phe Ser Asp His Phe His Val	
	365	370
		375
30	TCA TCC AAA AGT TCA TTA CAT ATG AAC TTC ATG TCC TCA CAC TCA AAC	1324
	Ser Ser Lys Ser Ser Leu His Met Asn Phe Met Ser Ser His Ser Asn	
	380	385
		390
35	AAC ATC CAC GCC CTC GAG CAA CTA AGG TGT ACA AAG AAC AAA GCA ACC	1372
	Asn Ile His Ala Leu Glu Gln Leu Arg Cys Thr Lys Asn Lys Ala Thr	
	395	400
		405

	AAT TTC TCT CAA TTT GTT AAG AAA ATG ATG GCT TCC TTG CCT TGT AGT	1420
	Asn Phe Ser Gln Phe Val Lys Lys Met Met Ala Ser Leu Pro Cys Ser	
	410 415 420	
5	TGT CAG TCT CAG AGT CCT TCC CCA TAT TTT GAC CAA TCT CTC TTC AGG	1468
	Cys Gln Ser Gln Ser Pro Ser Pro Tyr Phe Asp Gln Ser Leu Phe Arg	
	425 430 435 440	
10	TTT GAC GAG AAG CTT ATT TCA GCT ATT GAC CGC CAT AGA CAG TCT ACT	1516
	Phe Asp Glu Lys Leu Ile Ser Ala Ile Asp Arg His Arg Gln Ser Thr	
	445 450 455	
15	GAC CAT CCA ATC AAA TTC ATT TCT AGA AGA CAA CCC AAT ATC CTG AAA	1564
	Asp His Pro Ile Lys Phe Ile Ser Arg Arg Gln Pro Asn Ile Leu Lys	
	460 465 470	
	TTC AAA ATG AAG CCA GGA CCT GAA GCT GGC AGC ACA GAT GGG CGA ACT	1612
	Phe Lys Met Lys Pro Gly Pro Glu Ala Gly Ser Thr Asp Gly Arg Thr	
	475 480 485	
20	AAG AAG ATC TGT TCC TTC CTC TTC CAC CCA ATA TTA CCC CTT GCA CTT	1660
	Lys Lys Ile Cys Ser Phe Leu Phe His Pro Ile Leu Pro Leu Ala Leu	
	490 495 500	
25	TCT GTT CAA CAA ACC TTG TTT CTG CAG GCA TCA GTT GTA AAT ATC CAT	1708
	Ser Val Gln Gln Thr Leu Phe Leu Gln Ala Ser Val Val Asn Ile His	
	505 510 515 520	
30	TTT CGT CGA TAA TGTAAAAACT TAATTTATAT GTTACCGATT TGTTTATAAA	1760
	Phe Arg Arg *	
	TTTCTCTAAC AACCTCTAGA TTGAAATCAA CCTAGAAATC ACAAAATTCAT CATAACAGAC	1820
	CCGTAGATGC TAGTGTCTTT GACTTCTACA TTTCTTTGT TACAAGAATC AAACAAATGC	1880
	TTGATAGAGT GCCAAGACGG TTAGTATGGG TATAAGGATT AGTTCTCTG TAAGTTTTG	1940
35	TTACAGCTTC TCTTCTAACATT AATTGATGTA CATTAGATG TTAAAAAAAAA AAAAAAAAAA	2000

## (2) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 524 amino acids

(B) TYPE: amino acid

5 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Phe Lys Thr Asn Asn Val Thr Ala Arg Leu Phe Glu Arg Gln Ile  
1 5 10 15

10 Cys Thr Pro Ala Pro Gly Thr Ser Ile His Arg Ala Arg Arg Phe Tyr  
20 25 30

Glu Asn Val Val Pro Ser Tyr Thr Ile Tyr Asp Val Glu Cys Pro Asp  
35 40 45

His Ser Phe Arg Lys Phe Thr Asp Asp Gly Leu Tyr Phe Val Ser Phe  
15 50 55 60

Ser Arg Asn His Gln Asp Leu Val Val Tyr Arg Pro Thr Trp Leu Thr  
65 70 75 80

Phe Ser Cys Lys Glu Glu Asp Cys Asp Thr His Asp Leu Pro Leu Lys  
85 90 95

20 Ala Arg Lys Phe Glu Ser Phe Phe Thr Gln Leu Tyr Ser Val Thr Leu  
100 105 110

Ala Ser Ser Gly Glu Leu Ile Cys Lys Asp Phe Phe Leu Tyr Met Glu  
115 120 125

Ser Asn Gln Phe Gly Leu Phe Ala Thr Ser Thr Ala Gln Ile His Asp  
25 130 135 140

Ala Pro Pro Thr Gly Gly Ala Ile Gln Gly Val Pro Ser Val Glu Lys  
145 150 155 160

Ile Thr Phe His Leu Leu Arg Leu Val Asp Gly Ala Ile Leu Asp Glu  
165 170 175

30 Arg Val Phe His Asn Asp Tyr Val Asn Leu Ala His Ser Ile Gly Ala  
180 185 190

Phe Leu Tyr Asp Asp Leu Leu Ala Ile Val Ser Leu Arg Tyr Gln Arg  
195 200 205

Ile His Ile Leu Gln Ile Arg Asp Ser Gly Asp Leu Val Asp Val Arg  
35 210 215 220

	Ala Ile Gly Glu Phe Cys Arg Glu Asp Asp Glu Leu Phe Leu Asn Ser		
225	230	235	240
	Asn Ser Gln Val Leu Val Asn His Val Gly Asn Gly Phe His His Ser		
	245	250	255
5	Leu Pro Gln Ser Glu Thr Ser Phe Leu Ser Gly Ile Lys Gln Arg Leu		
	260	265	270
	Leu Ser Tyr Ile Phe Arg Gly Ile Trp Asn Glu Ala Asp Gln Thr Met		
	275	280	285
10	Arg Val Gln Cys Leu Lys Lys Phe Tyr Phe His Phe Gln Asp Tyr		
	290	295	300
	Ile Asp Leu Ile Ile Trp Lys Val Gln Phe Leu Asp Arg His His Leu		
	305	310	315
	Leu Ile Lys Phe Gly Ser Val Asp Gly Gly Val Ser Arg Asn Ala Asp		
	325	330	335
15	Ile His Pro Ser Phe Phe Ala Val Tyr Asn Met Glu Thr Thr Glu Ile		
	340	345	350
	Val Ala Phe Tyr Gln Asn Ser Ala Asp Glu Leu Tyr Phe Leu Phe Glu		
	355	360	365
	Leu Phe Ser Asp His Phe His Val Ser Ser Lys Ser Ser Leu His Met		
20	370	375	380
	Asn Phe Met Ser Ser His Ser Asn Asn Ile His Ala Leu Glu Gln Leu		
	385	390	395
	Arg Cys Thr Lys Asn Lys Ala Thr Asn Phe Ser Gln Phe Val Lys Lys		
	405	410	415
25	Met Met Ala Ser Leu Pro Cys Ser Cys Gln Ser Gln Ser Pro Ser Pro		
	420	425	430
	Tyr Phe Asp Gln Ser Leu Phe Arg Phe Asp Glu Lys Leu Ile Ser Ala		
	435	440	445
	Ile Asp Arg His Arg Gln Ser Thr Asp His Pro Ile Lys Phe Ile Ser		
30	450	455	460
	Arg Arg Gln Pro Asn Ile Leu Lys Phe Lys Met Lys Pro Gly Pro Glu		
	465	470	475
	Ala Gly Ser Thr Asp Gly Arg Thr Lys Lys Ile Cys Ser Phe Leu Phe		
	485	490	495
35	His Pro Ile Leu Pro Leu Ala Leu Ser Val Gln Gln Thr Leu Phe Leu		
	500	505	510

Gln Ala Ser Val Val Asn Ile His Phe Arg Arg \*  
515 520

## (2) INFORMATION FOR SEQ ID NO: 3:

## 5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CATCAACACT GCCAAAC

17

## (2) INFORMATION FOR SEQ ID NO: 4:

## 15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

20 GAAAGCAGCC GTTGCT

16

## (2) INFORMATION FOR SEQ ID NO: 5:

## 25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

AGTTCATCAT CTTCACGGC

19

30

## (2) INFORMATION FOR SEQ ID NO: 6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:  
GTATGATTCA CTAGTTAAC GCTGCTGAAA G

31

(2) INFORMATION FOR SEQ ID NO: 7:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CCCATACTAG TCGTCTTGGC ACTCTATCAA G

31

(2) INFORMATION FOR SEQ ID NO: 8:

15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

20 GAAGGTAATT TTATATTAAA CATAGAATAG A

31

(2) INFORMATION FOR SEQ ID NO: 9:

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GTGATTCTA GGTTGATTTC AATCTAGA

28

30

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CAAATCGGTA ACATAT

16

(2) INFORMATION FOR SEQ ID NO: 11:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GGGGAAGCTT TTTCACGAAA TCGGCCCTTA

30

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 33 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

20 CCCGGATCCT TCTTTGCAC TGTGAATGAT TAG

33

## CLAIMS

1. Nucleic acid comprising a nucleotide sequence encoding a protein, or functional portions thereof, which, if altered, is responsible for the light hypersensitive mutant phenotype in *Solanum lycopersicum* plants, said phenotype comprising a reduced growth of the plant associated with high levels of carotenoids and/or chlorophylls and/or flavonoids.

2. Nucleic acid according to claim 1 encoding a tomato protein homologous to at least one of the *Arabidopsis* COP/DET/FUS family proteins, provided that it is responsible for the light hypersensitive mutant phenotype.

3. Nucleic acid according to claim 2 comprising the nucleotide sequence encoding the TDET1 (HP-2) protein, or functional portions thereof.

4. Nucleic acid according to claim 3 comprising a nucleotide sequence encoding the protein having the amino acid sequence of SEQ ID No. 2, or functional portions thereof.

5. Nucleic acid according to claim 4 comprising a nucleotide sequence comprised in SEQ ID No. 1.

6. Nucleic acid according to claim 5 wherein the nucleotide sequence comprised in SEQ ID No. 1 is the sequence from nt. 149 to nt. 1720, or portions thereof.

7. Nucleic acid comprising a nucleotide sequence complementary to SEQ ID No. 1 or portions thereof.

8. Nucleic acid according to claim 3 or 4 comprising a mutation which is able to induce the light hypersensitive phenotype.

30 9. Nucleic acid according to claim 8 wherein the SEQ ID No. 1 comprises at least a T as a substitute for C at position 1640.

10. Nucleic acid according to claim 8 wherein the SEQ ID No. 1 comprises at least a nucleotide deletion from nt. 1581 to nt. 1589.

5 11. Expression vector comprising the nucleic acid according to any one of preceding claims under the control of a promoter that is active in plants.

12. Vector according to claim 11 wherein said promoter is selectively active only in a few plant organs.

10 13. Vector according to claim 12 wherein said promoter is active in the fruits.

14. Vector according to any one of preceding claims 11-13 which is able to control the transcription of an antisense RNA.

15 15. Vector according to claim 14 wherein said vector is the pBIN-E8-HP2-AS1 vector or the pBIN-E8-HP2-AS1 vector.

20 16. Use of vector according to any one of preceding claims 11-15 to produce transgenic plants containing the nucleic acid according to any one of preceding claims 1-10, under the control of specific regulatory sequences, preferably in selected organs of plants.

25 17. Use of vector according to claim 16 wherein said transgenic plant belongs to pepper, eggplant, soybean, grape, melon, rice, carrot, spinach, citrus, pomaceae or ornamental species.

18. Transgenic plant obtainable by the use of transforming vector according to any one of the preceding claims 11-15.

30 19. Transgenic plant according to claim 18 belonging to pepper, eggplant, soybean, grape, melon, rice, carrot, spinach, citrus, pomaceae and ornamental species.

20. Protein, or portions thereof, which, if altered, is responsible for the light hypersensitive phenotype in *Solanum lycopersicum* plants.

5 21. A protein according to claim 20 homologous with at least one of the *Arabidopsis* COP/DET/FUS family proteins, whose alteration is responsible for a light hypersensitive phenotype.

22. Protein according to claim 21 comprising the amino acid sequence of SEQ ID No. 2, or portions thereof.

10 23. Protein according to claim 22 comprising an alteration to the amino acid sequence of SEQ ID No. 2, or portions thereof, which are able to induce a light hypersensitive phenotype.

15 24. Protein according to claim 23 comprising at least an alteration in the SEQ ID No. 1 C-terminal portion.

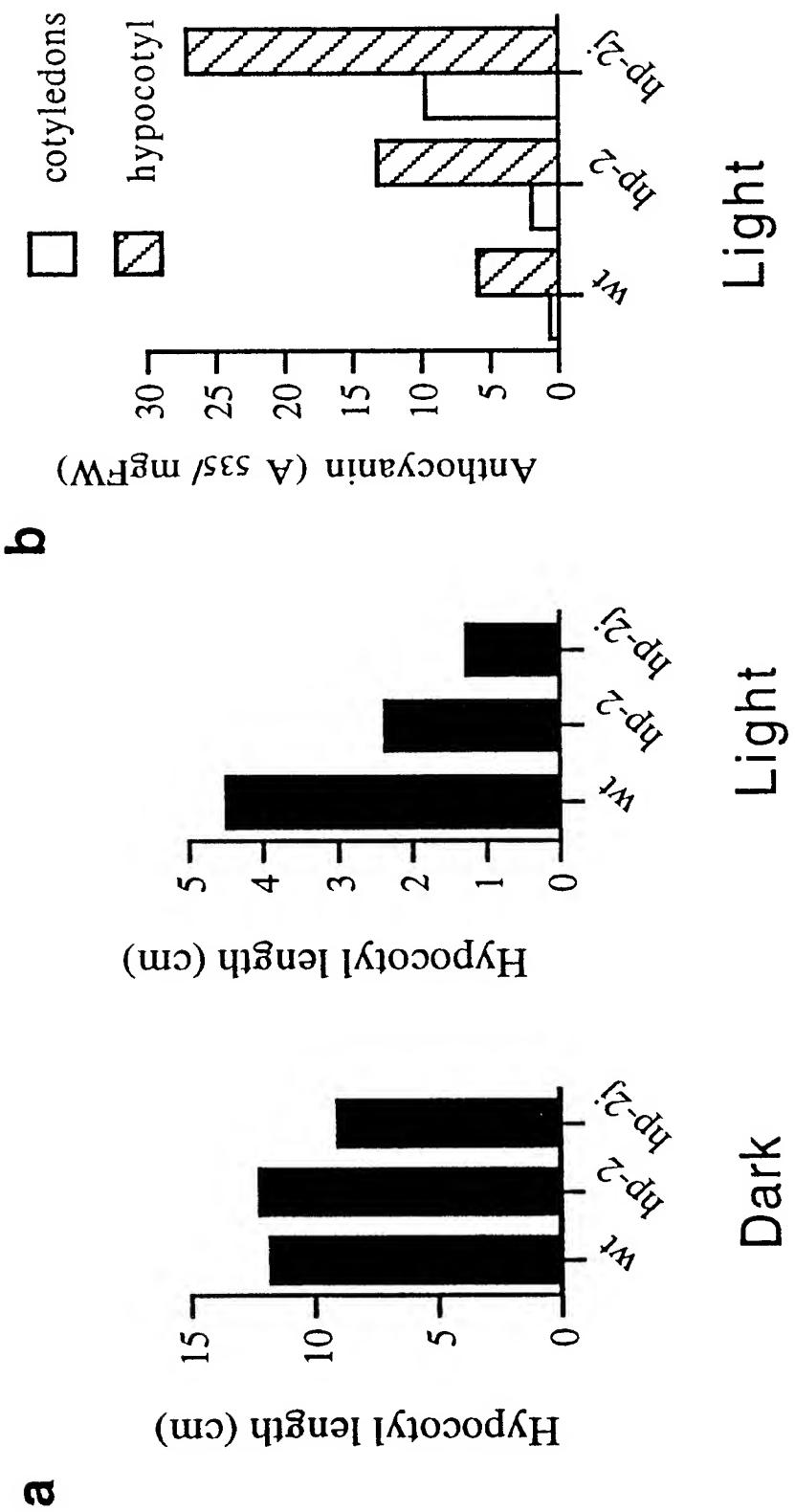
25. Protein according to claim 24 wherein said alteration in the C-terminal portion comprises a substitution of proline at 498 position.

20 26. Protein according to claim 25 wherein said substitution comprises a serine as a substitute for proline.

27. Protein according to claim 24 wherein said alteration in the C-terminal portion comprises a deletion of at least one amino acid in the second NLS domain.

25 28. Protein according to claim 27 wherein said deletion comprises at least three amino acids.

29. Protein according to claim 28 wherein said deletion comprising at least three amino acids is from aa. 478 to aa. 480.

**Fig. 1**

## 2 / 10

1 cttcccttctt agactttatc gatcctaatt cgagccctcc tttttcaat  
 51 caattatcaa ttttagtccta ctgcgatttt gatatgtatg attcacaatt  
 101 ttaatgctgc tgaaaagcaat tatataaaaag ctgaaacatt ttgcactgAT  
 151 GTTCAAAACT AACAAATGTTA CCGCCAGGCT TTTTGAGCGC CAGATTGCA  
 201 CCCCTGCTCC TGGCACCAAGC ATCCATCGTG CCAGAAAGATT TTATGAGAAC  
 251 GTTGTACCAA GTTATACCAT ATACCGATGTT GAATGTCCCG ACCATTCAATT  
 301 TCGCAAGTTC ACGGATGACG GTCTATATTT TGTAAGTTTC AGCCGAAACC  
 351 ATCAGGATCT GGTTGTTAT AGACCAACAT GGCTGACATT TTCCTGCAAA  
 401 GAAGAAGATT GTGATACTCA TGATCTTCCT TTGAAAGCTA GAAAGTTGA  
 451 GAGCTTCTTC ACACAGTTGT ACAGTGTAC TCTTGCTTCT AGTGGGGAAC  
 501 TTATATGCAA AGATTCTTT CTCTATATGG AGAGCAACCA ATTTGGACTC  
 551 TTTGCAACTT CAACTGCACA AATTCAATGAT GCACCTCCTA CTGGAGGGGC  
 601 AATTCAAGGGA GTCCCTTCAG TTGAAAAAAT AACTTCCAC CTTTGAGGTT  
 651 TGGTGGATGG AGCTATACTT GACGAAAGGG TTTTCCACAA TGATTATGTT  
 701 AATTGGCAC ATAGCATTGG TGCTTCTTG TATGATGATT TGCTTGCTAT  
 751 AGTGTCTCTT CGTTATCAA GAATACACAT CCTTCAGATC AGAGATTCTG  
 801 GAGATCTTGT TGATGTACGA GCAATTGGGG AATTCTGCCG TGAAGATGAT  
 851 GAACTTTTC TCAATTCCAA TTCCCAGGTG CTTGTAAATC ATGTTGGAAA  
 901 TGGTTTCAT CATACTCTGC CTCAATCAGA AACTTCTTTC CTGAGCGGTA  
 951 TAAAGCAACG GCTGCTTCA TATATATTTG GAGGTATATG GAATGAAGCT  
 1001 GACCAAACCA TGAGAGTGCA GTGCCTGAAG AAGAAGTTTT ACTTCCACTT  
 1051 TCAAGATTAC ATTGACTTGA TTATCTGGAA GGTGCAGTTT TTGGACCGAC  
 1101 ATCACCTGTT GATCAAGTTT GGCAGTGTIG ATGGTGGGGT ATCCCGAAAT  
 1151 GCTGACATCC ATCCTTCTTT TTTTGCTGTT TACAATATGG AGACTACTGA  
 1201 AATTGTTGCA TTTTATCAGA ACTCAGCCGA TGAGCTTTAT TTCTTGTTG  
 1251 AGCTGTTCAAG CGACCATTTC CACGTTTCAT CCAAAAGTTTC ATTACATATG  
 1301 AACTTCATGT CCTCACACTC AAACAACATC CACGCCCTCG AGCAACTAAG  
 1351 GTGTACAAAG AACAAAGCAA CCAATTCTC TCAATTGTT AAGAAAATGA  
 1401 TGGCTTCCTT GCCTGTAGT TGTCAGTCTC AGAGTCCTTC CCCATATTT  
 1451 GACCAATCTC TCTTCAGGTT TGACGAGAAG CTTATTTCAG CTATTGACCG  
 1501 CCATAGACAG TCTACTGACC ATCCAATCAA ATTCAATTCT AGAAGACAAC  
 1551 CCAATATCCT GAAATTCAA ATGAAGCCAG GACCTGAAGC TGGCAGCACA  
 1601 GATGGGGCAA CTAAGAAGAT CTGTTCTTC CTCTTCCACC CAATATTACC  
 1651 CCTTGCACTT TCTGTTCAAC AACCTTGTT TCTGCAGGCA TCAGTTGTAA  
 1701 ATATCCATTTC TCGTCGATAA tgtaaaaaact taatttatat gttaccgatt  
 1751 tgtttataaa tttctctaatt aacctctaga ttgaaatcaa cctagaaatc

Fig. 2

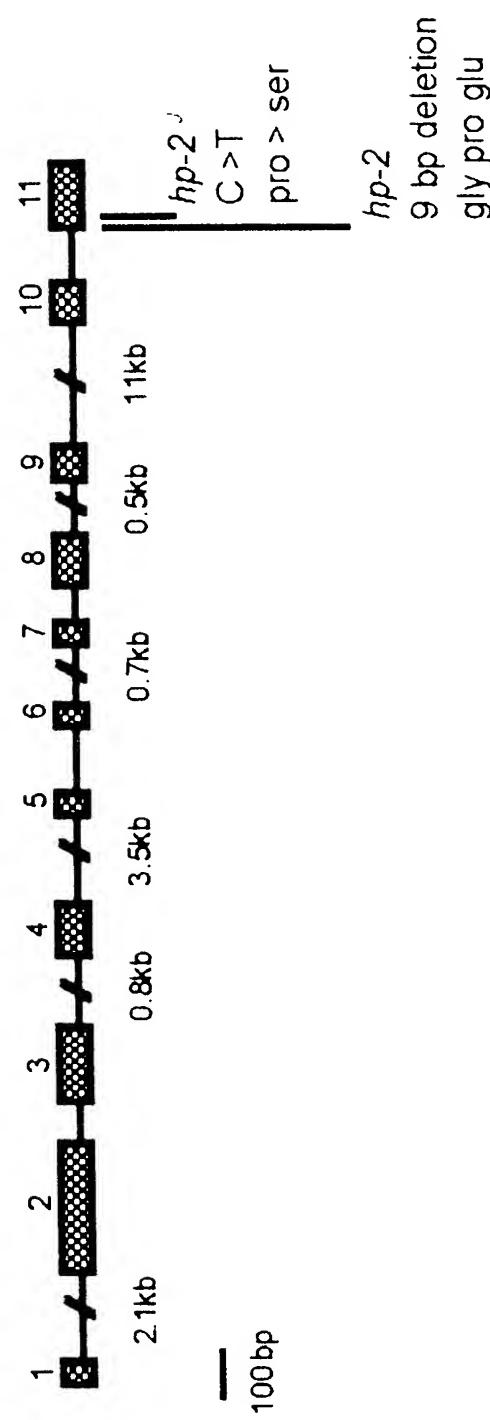
1801 acaaattcat cataacagac ccgttagatgc tagtgtcttt gacttctaca  
1851 ttttcttgt tacaagaatc aaacaaatgc ttgatagagt gccaaagacgg  
1901 ttagtatggg tataaggatt agttcttctg taagtttttg ttacagcttc  
1951 tcttctaatt aattgatgta cattcagatg tttaaaaaaaaaaaaaaa

**Fig. 2'**

1 MFKITNNVTAR LFERQICTPA PGTSIHRARR FYENVVPSYT IYDVECPDHS  
51 FRKFTDDGLY FVSFSRNHQD LVVYRPTWLT FSCKEEDCDT HDLPLKARKF  
101 ESFFTQLYSV TLASSGELIC KDFFLYMESN QFGLFATSTA QIHDAPPTGG  
151 AIQGVPSVEK ITFHLLRLVD GAILDERVFH NDYVNLAHSI GAFLYDDLLA  
201 IVSLRYQRIH ILQIRDSGDL VDVRAIGEFC REDDEFLNS NSQVLVNHVG  
251 NGFHHSLPQS ETSFLSGIKQ RLLSYIFRGI WNEADQTMRV QCLKKKFYFH  
301 FQDYIDLIIW KVQFLDRHHL LIKFGSVDGG VSRNADIHPS FFAVYNMETT  
351 EIVAFYQNSA DELYFLFELF SDHFHVSSKS SLHMNFMSSH SNNIHALEQL  
401 RCTKNKATNF SQFVKKMMAS LPCSCQSQSP SPYFDQSLFR FDEKLISAID  
451 RHRQSTDHPI KFISRRQPNI LKFKMKPGPE AGSTDGRTKK ICSFLFHPIL  
501 PLALSVQQTL FLQASVVIH FRR

**Fig. 3**

Fig. 4

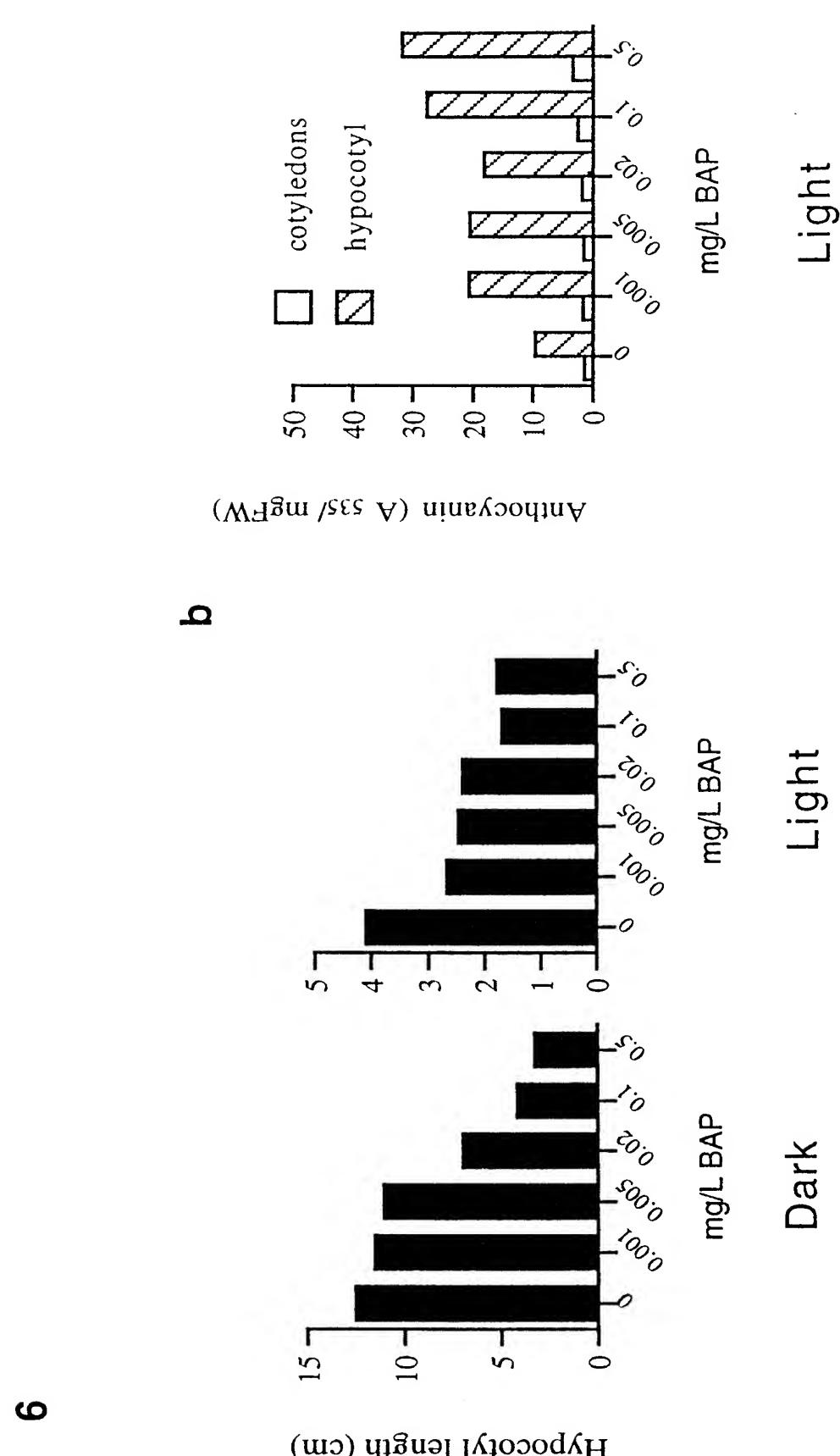
**a****b**

*wild type* ... AAG CCA G [GTACGG...TATCAG] GA CCT GAA GCT GGG ...  
 K P G P E A G

*hp-2* ... AAG CCA G [GTACGG...TATCTG] GA CCT GAAG ] CT GGG ...  
 K P \* A G

Fig. 5

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**Fig. 6**

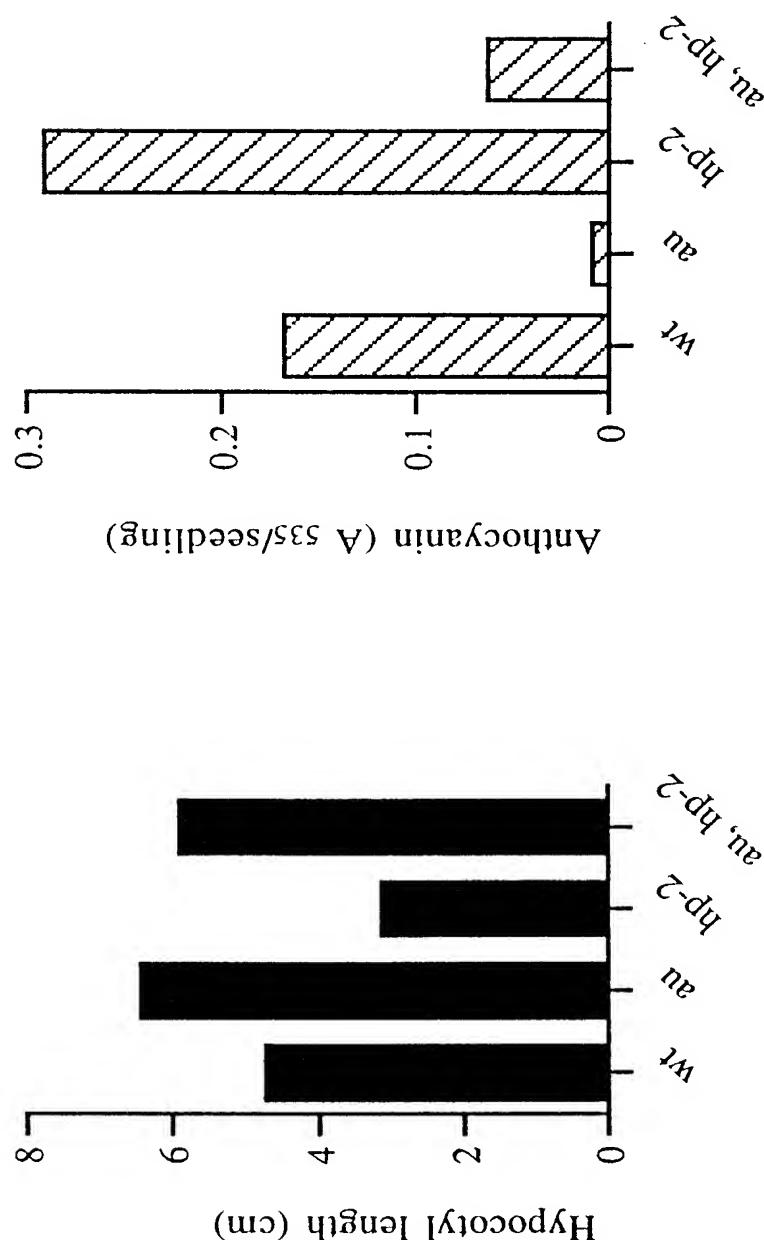
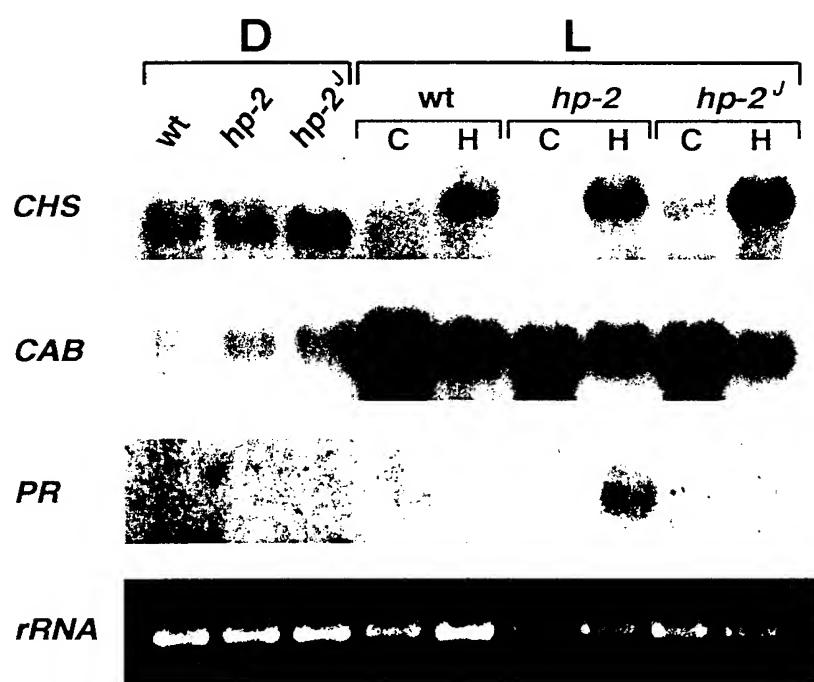
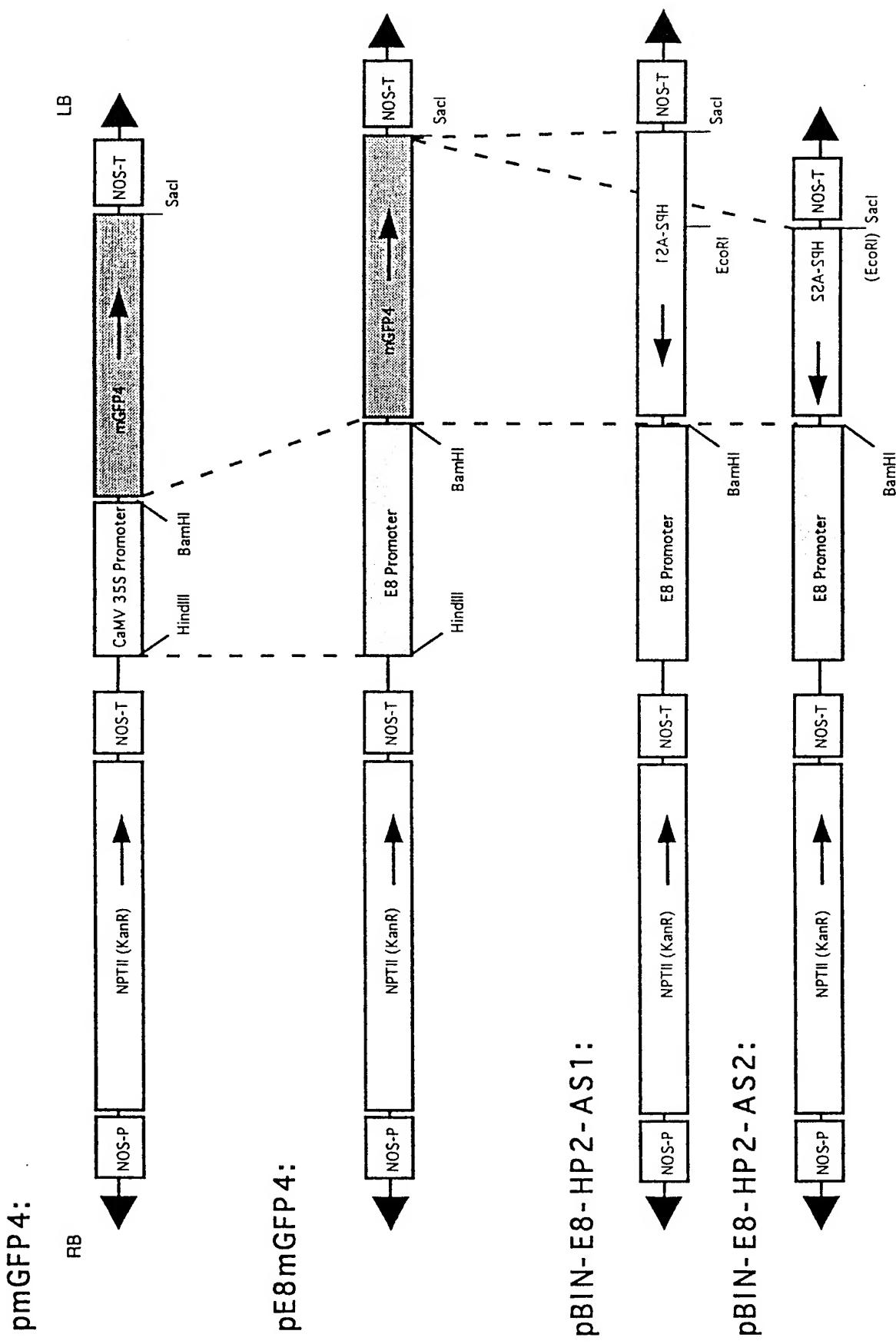


Fig. 7



**Fig. 8**

**Fig. 9**

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/IT 98/00350

**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC 6 C12N15/29 C12N15/82 C07K14/415 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
 IPC 6 C12N C07K A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FRAY R G ET AL: "CONSTITUTIVE EXPRESSION OF A FRUIT PHYTOENE SYNTHASE GENE IN TRANSGENIC TOMATOES CAUSES DWARFISM BY REDIRECTING METABOLITES FROM THE GIBBERELLIN PATHWAY" PLANT JOURNAL, vol. 8, no. 5, November 1995, pages 693-701, XP002043131 see the whole document --- TANKSLEY, S.D., ET AL.: "High density molecular linkage maps of the tomato and potato genomes" GENETICS, vol. 132, December 1992, pages 1141-1160, XP002097980 see page 1142, left-hand column; figure 1 --- -/-/	1,11
X		1-3

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

\* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

6 April 1999

16/04/1999

Name and mailing address of the ISA  
 European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl  
 Fax: (+31-70) 340-3016

Authorized officer

Maddox, A

**INTERNATIONAL SEARCH REPORT**

International Application No
PCT/IT 98/00350

**C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT**

Category <sup>a</sup>	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	GANAL, M.W., ET AL.: "CT151.KS Tomato leaf cDNA from cv. VFNT cherry Lycopersicon esculentum cDNA clone CT151" EMBL ACCESSION NO. AA824865, 20 February 1998, XP002097199 see the whole document ---	1-7
Y	VAN TUINEN, A., ET AL.: "The mapping of phytochrome genes and photomorphogenetic mutants of tomato" THEOR APPL GENET, vol. 94, 1997, pages 115-122, XP002097981 see page 117 - page 118 ---	1-10
Y	KERCKHOFFS, L.H.J., ET AL.: "Photocontrol of anthocyanin biosynthesis in tomato" J PLANT RES, vol. 110, 1997, pages 141-149, XP002097982 see page 146, right-hand column - page 147, left-hand column, line 5 ---	1-10
A	KERCKHOFFS, L.H.J., ET AL.: "Physiological characterization of exaggerated photoresponse mutants of tomato" J. PLANT PHYSIOL., vol. 150, 1997, pages 578-587, XP002097983 see the whole document ---	1-29
A	YEN H. C., ET AL.: "The tomato high-pigment (hp) locus maps to chromosome 2 and influences plastome copy number and fruit quality" THEORETICAL AND APPLIED GENETICS, (NOV 1997) VOL. 95, NO. 7, PP.1069-1079. , XP002097198 see the whole document ---	1
A	PEPPER, A., ET AL.: "DET1, a negative regulator of light-mediated development and gene expression in <i>Arabidopsis</i> , encodes a novel nuclear-localized protein" CELL, vol. 78, 1994, pages 109-116, XP002097200 see the whole document & EMBL ACCESSION NO. L33695, 20 July 1994, ---	1-10, 20-29
A	BOYLAN, M.T., ET AL.: "Oat phytochrome is biologically active in transgenic tomatoes" THE PLANT CELL, vol. 1, 1989, pages 765-773, XP002097201 see the whole document ---	11-19
	-/-	

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/IT 98/00350

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MCNELLIS, T.W., ET AL.: "Expression of an N-terminal fragment of COP1 confers a dominant-negative effect on light-regulated " THE PLANT CELL, vol. 8, September 1996, pages 14901-1503, XP002097202 see the whole document ----	11-19
A	MCNELLIS, T.W., ET AL.: "Overexpression of Arabidopsis COP1 results in partial suppression of light-mediated development: evidence for a light-inactivatable repressor of photomorphogenesis" THE PLANT CELL, vol. 6, 1994, pages 1391-1400, XP002097203 see the whole document ----	11-19
A	WO 97 20941 A (UNIV KINGSTON ;KO KENTON (CA); KO ZDENKA W (CA); LABATE CARLOS (BR) 12 June 1997 see page 42, line 5 - line 28 ----	11-19
A	WO 97 39112 A (LI JIANMING ;CHORY JOANNE (US); SALK INST FOR BIOLOGICAL STUDI (US) 23 October 1997 see the whole document ----	11-19
A	WO 96 13149 A (AMOCO CORP) 9 May 1996 see page 36, line 1 - line 7; claims 1-4,9-11 ----	11-19
A	WO 97 14807 A (SEMINIS VEGETABLES) 24 April 1997 see page 17, line 18 - line 25 ----	11-19
T	MUSTILLI, A.C., ET AL.: "Phenotype of the tomato high pigment-2 mutant is caused by a mutation in the tomato homolog of deetiolated1" THE PLANT CELL, vol. 11, no. 2, February 1999, XP002097204 see the whole document & EMBL ACCESSION NO. AJ222798, 18 December 1998, & EMBL ACCESSION NO. AJ224356, 8 February 1999, & EMBL ACCESSION NO. AJ224357, 8 February 1999, -----	1-10, 20-29

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr	nal Application No
	PCT/IT 98/00350

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
WO 9720941	A 12-06-1997	AU CA CN EP	7688896 A 2239305 A 1203631 A 0871751 A		27-06-1997 12-06-1997 30-12-1998 21-10-1998
WO 9739112	A 23-10-1997	AU EP	2728197 A 0904354 A		07-11-1997 31-03-1999
WO 9613149	A 09-05-1996	US AU AU CA CN EP JP NO PL	5618988 A 697358 B 3970195 A 2203815 A 1172416 A 0792352 A 10509309 T 971945 A 319788 A		08-04-1997 01-10-1998 23-05-1996 09-05-1996 04-02-1998 03-09-1997 14-09-1998 27-06-1997 01-09-1997
WO 9714807	A 24-04-1997	AU EP	5377896 A 0873413 A		07-05-1997 28-10-1998